

FIG._1A

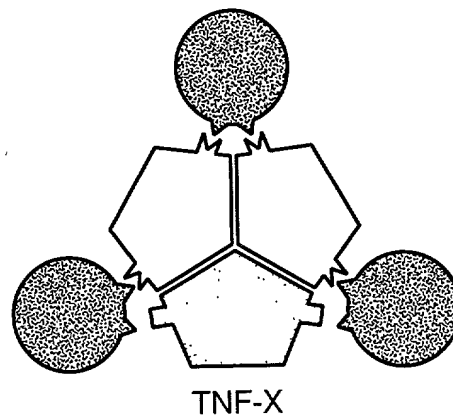


FIG._1B



200610-0827500

TNF-TNFR TRIMER COMPLEX

SIDE VIEW

TOP VIEW



FIG.-2

1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100

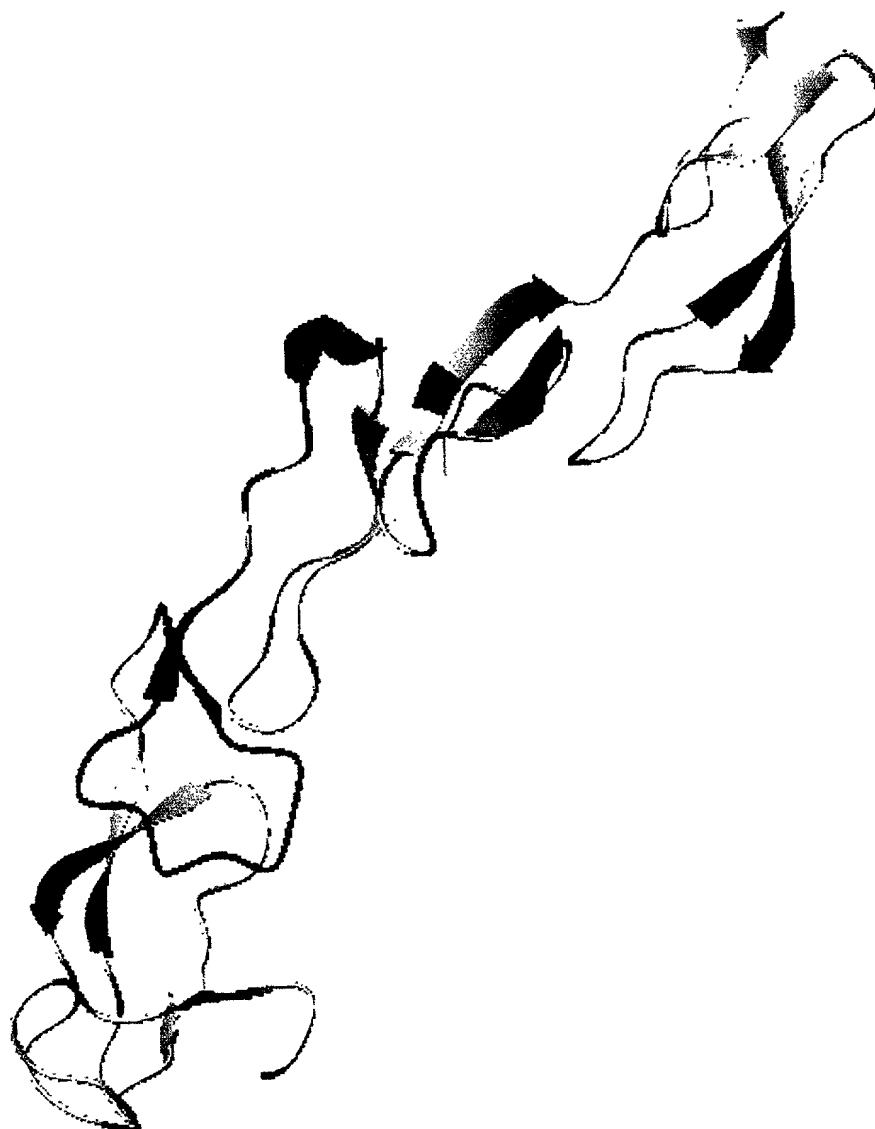
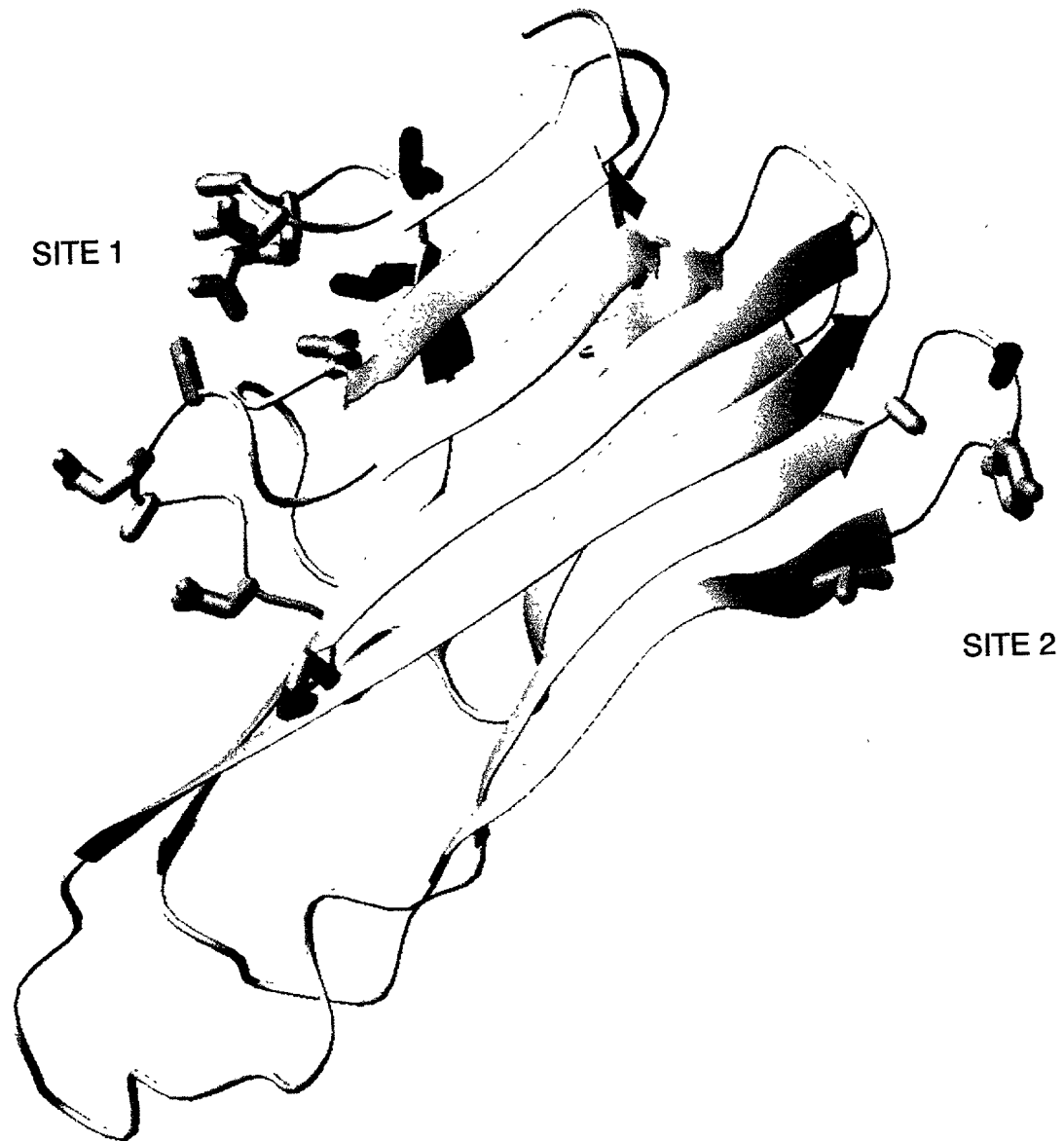
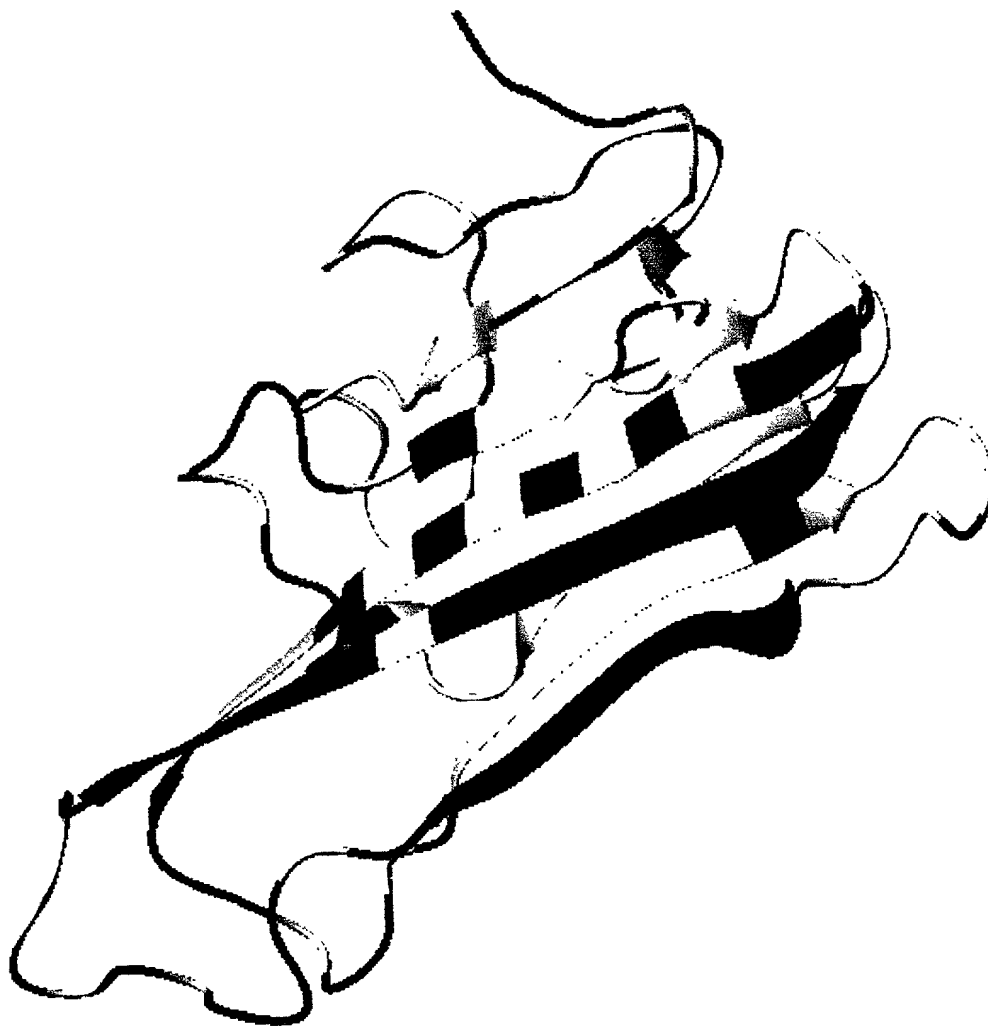


FIG. 3

TNF α BINDING SITES**FIG._4**

TNF α TRIMER INTERFACE**FIG._5**

1 atgcaccacc accaccacca cgtacgctcc tectcccga ctcgctccga caaacccgta
 61 gctcacgtag tagctaacc gcaggctgaa ggtcagctgc agtggtgaa ccgccgcgt
 121 aacgctctgc tggctaaccg ttagaactg cgcgacaacc agctggtagt accgtccgaa
 181 ggtctgtacc tgatctactc ccaggctactg ttcaaaggctc aggggtgtcc gtccactcac
 241 gtactgtgta ctcacactat ctcccgcatc gctgtatcct accagactaa agtaaacctg
 301 ctgtccgcta tcaaattccc gtgtcagcgc gaaactccgg aagggtgctga agctaaaccg
 361 tggtagaac cgatctacct ggggtggtgta ttccagctgg aaaaagggtga ccgcctgtcc
 421 gctgaaatca accgcccggga ctacctggac ttcgctgaat ccggtcaggt atacttcggt
 481 atcatcgctc tgtga

FIG._6A

1 MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRRA NALLANGVEL RDNQLVVPSE
 61 GLYLIYSQVL FKGQGCPSTH VLLTHTISRI AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP
 121 WYEPIYLGGV FQLEKGDRLS AEINRPDYLD FAESGQVYFG IIAL

FIG._6B

Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants created
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

ALSO MADE DOUBLE MUTANTS K65E/D143K, K65E/D143R, K65D/D143K AND K65D/D143R

FIG._7

Cell for 63643600

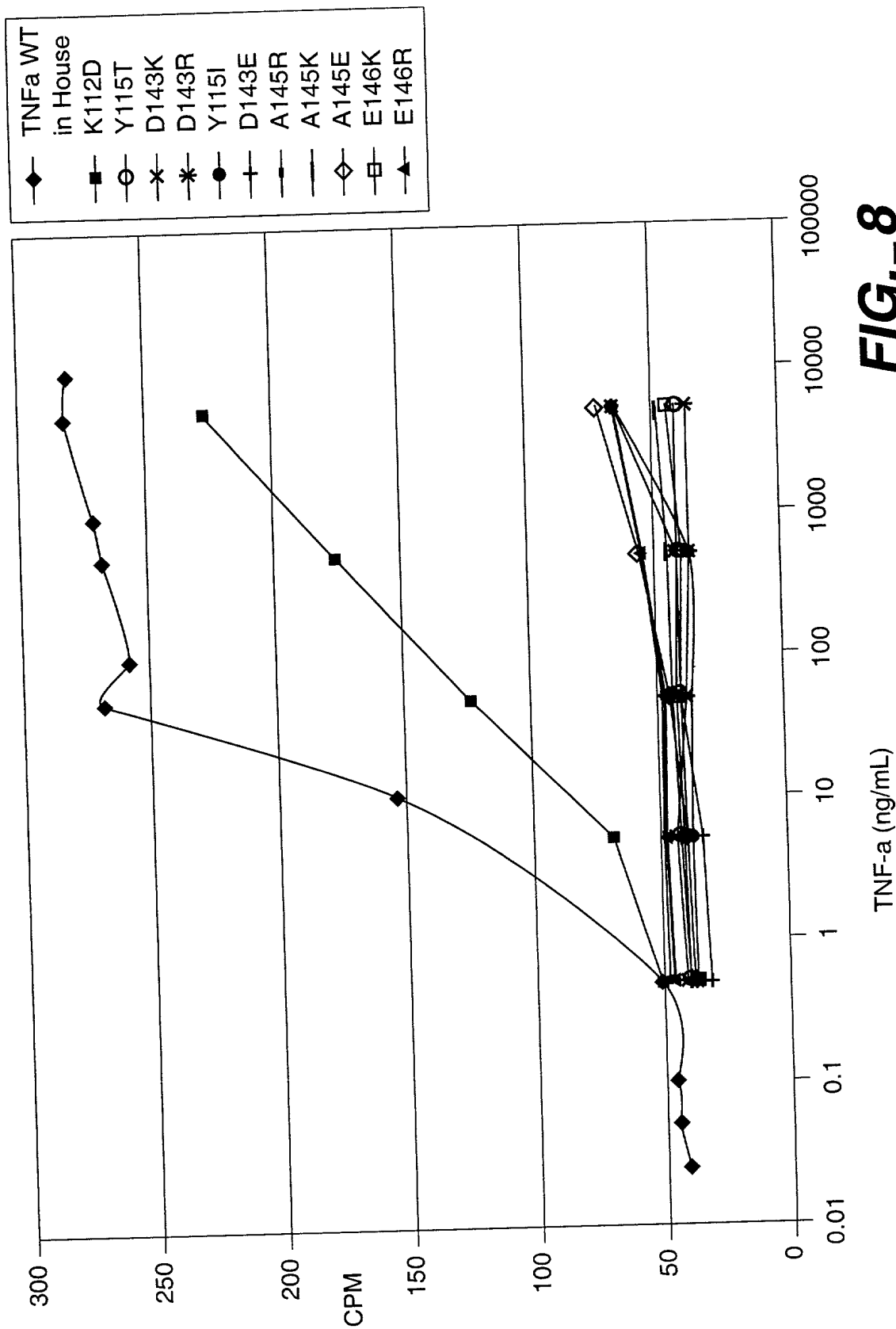


FIG._8

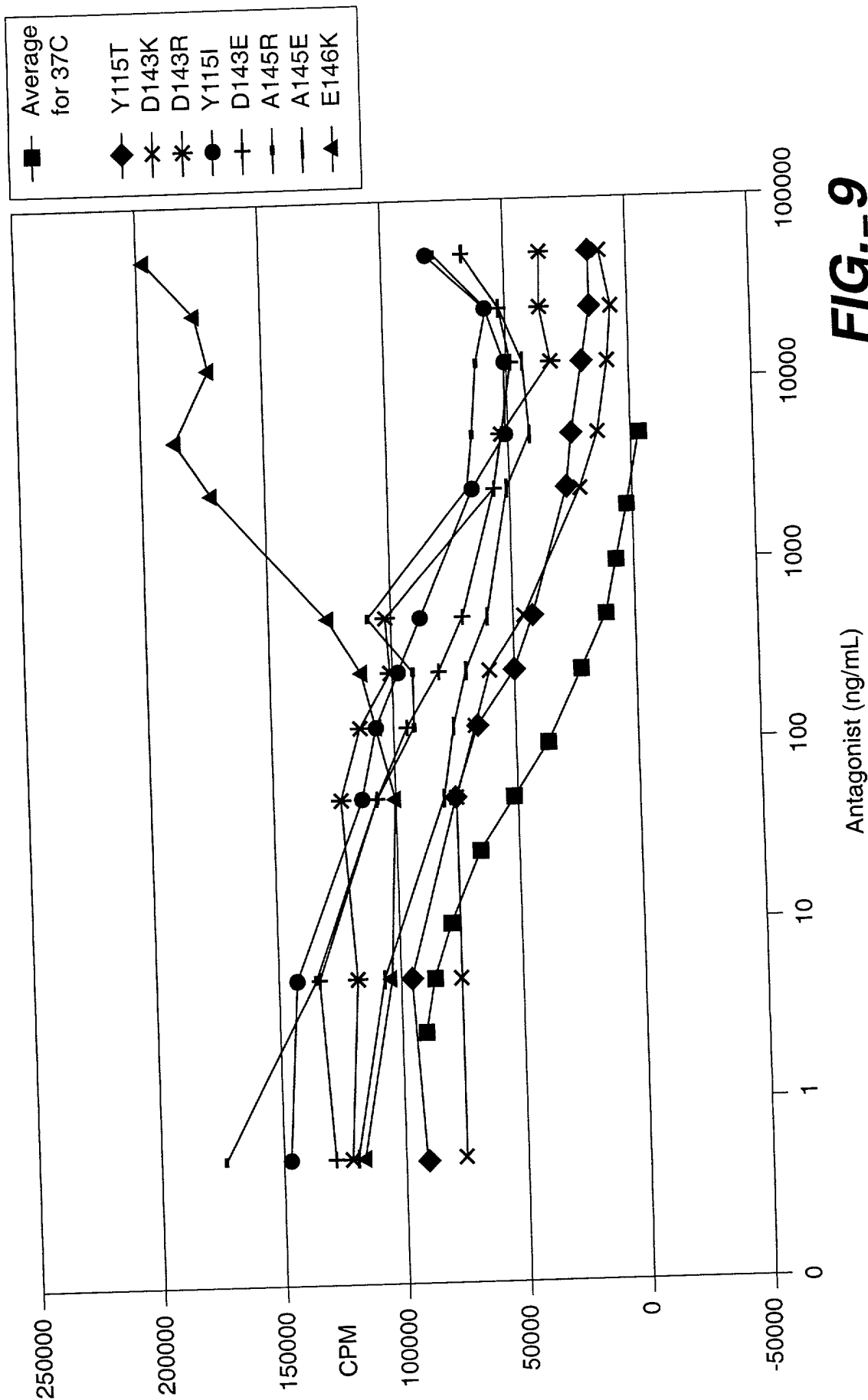


FIG. 9

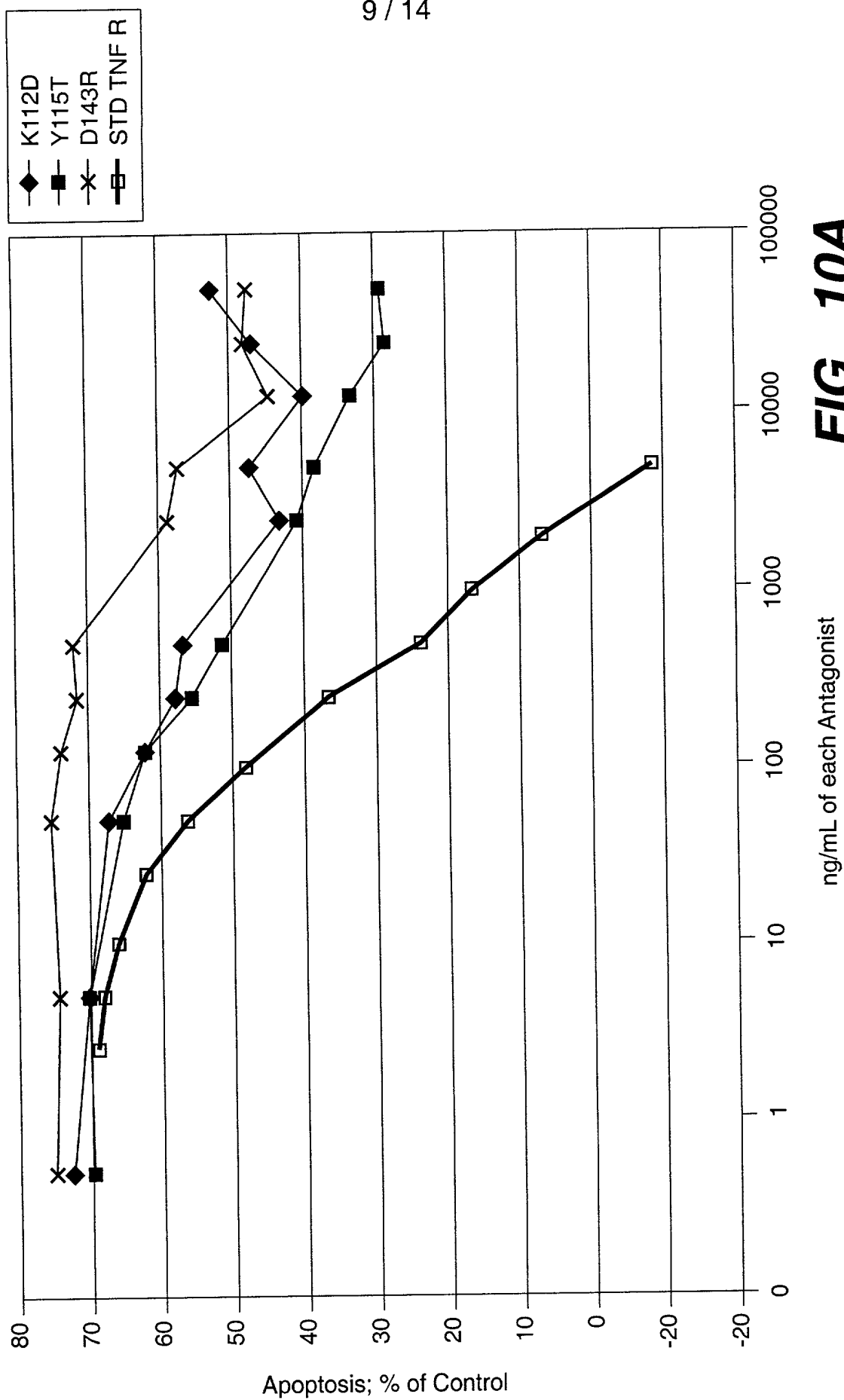
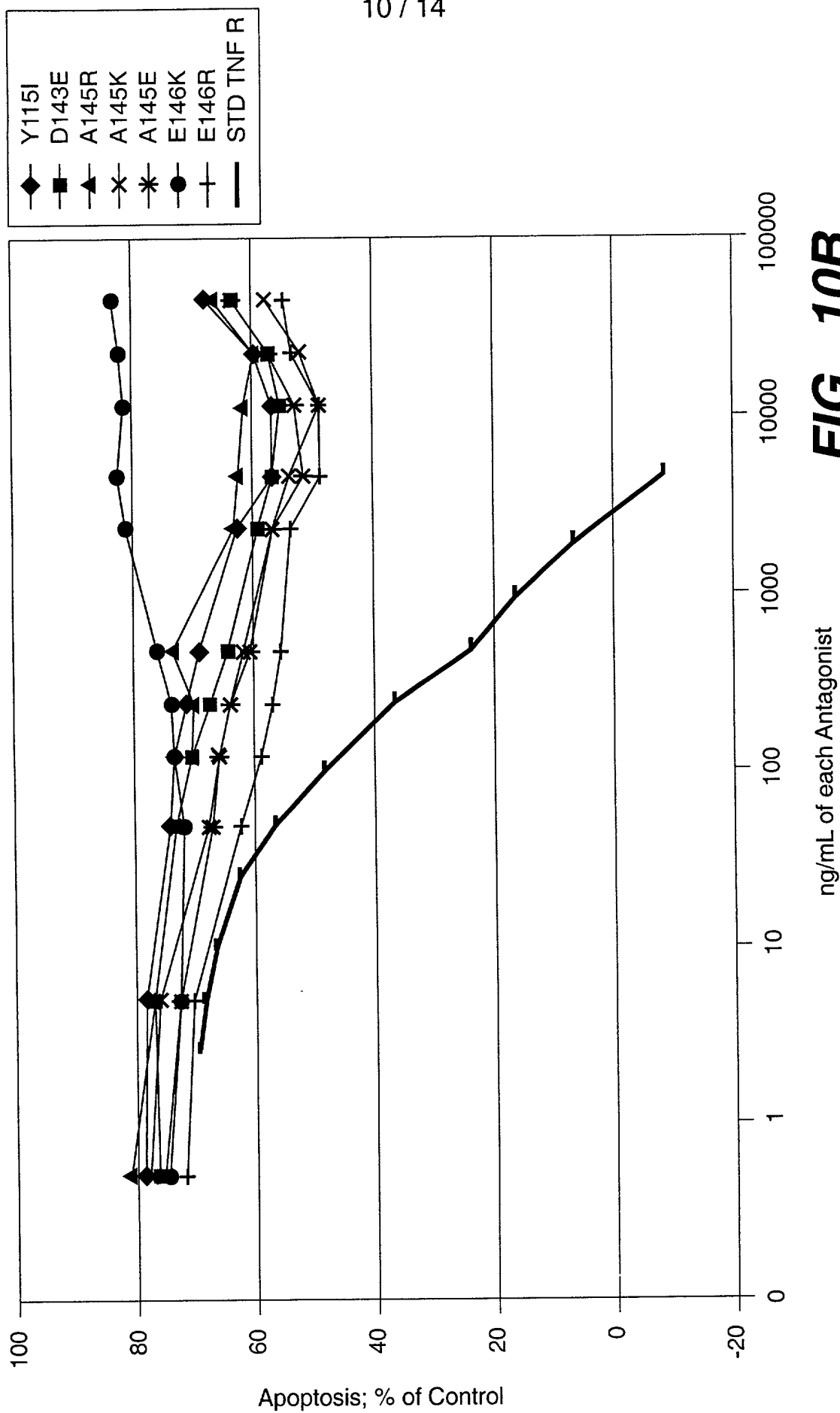


FIG. 10A



WT	PDA Relative Probability Distribution																			
Q21	R1000																			
N30	D1000																			
R31	I1000																			
R32	H1000																			
A33	E1000																			
A35	S1000																			
K65	R585	D146	K110	T42	H31	M27	W15	I15	Q10	S9	N9	V1								
G66	Q813	K187																		
Q67	D623	W209	Y83	R43	K41	S1														
A111	R959	E41																		
K112	K1000																			
Y115	Q230	K154	E116	N84	Y81	R72	F69	H43	M39	L36	I26	W25	D11	T8	S6					
D140	D1000																			
L143	D680	E130	N110	Q33	S29	R12	K6													
F144	F695	N305																		
A145	R456	D196	K124	N76	H67	T43	Q25	E9	Y1	M1	S1	F1								
E146	N489	K377	R111	D12	S10	E1														
S147	R1000																			

FIG._ 11

TRAF2(310-) DQDKIEALSSKVQQQLERSIGLKDLAMADLEQKVLEMEA STYDG

FIG._12A

TRAF3(374-) VARNTGLLESQLSRHDQMLSVHDIRLADMDLRFQVLET ASYNG

FIG._12B

TRAF5(343-) NDQRLAVLEEETNKHDTHINIHKAQLSKNEERFKLLEG TCYNG

FIG._12C

TRAF1(225-) DRERILSLEQRVVVELQQTLAQKDQALGKLEQSLRLMEE ASFDG

FIG._12D

TRAF6(309-) QDHQIRELTAKMETQSMYVSELKRTIRTLEDKVAEIEA QQCNG

FIG._12E

TRAF4(201-) -----CALVSRQRQELQELRRELEELSV GS-DG

FIG._12F

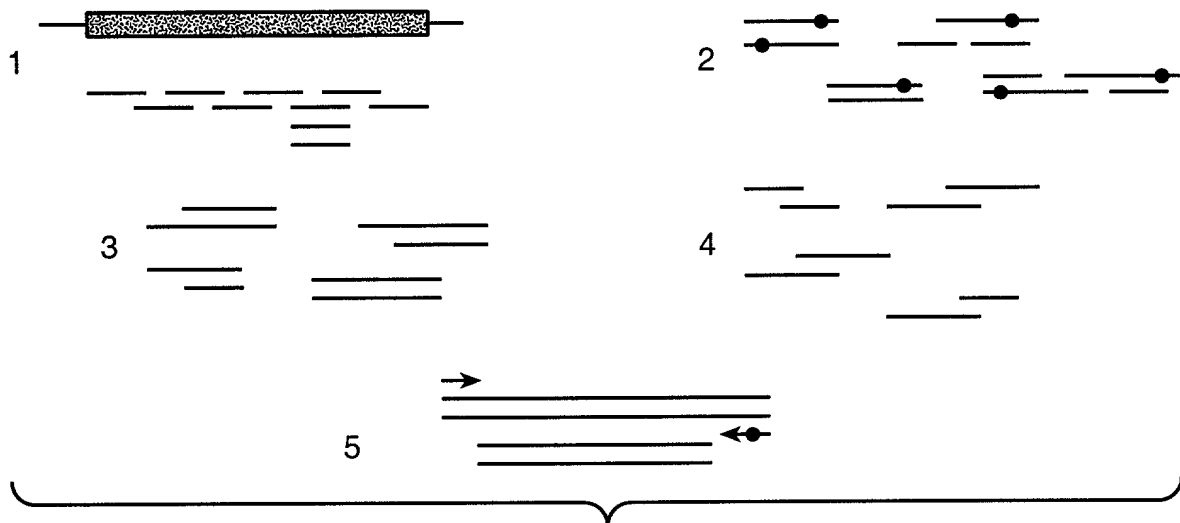


FIG._13

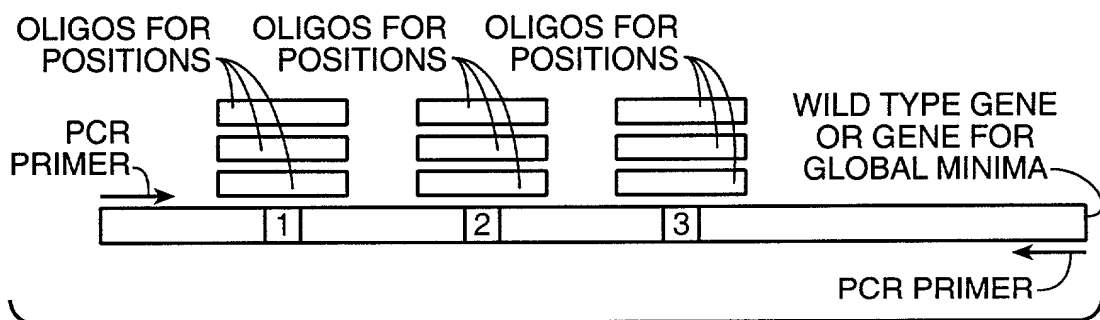
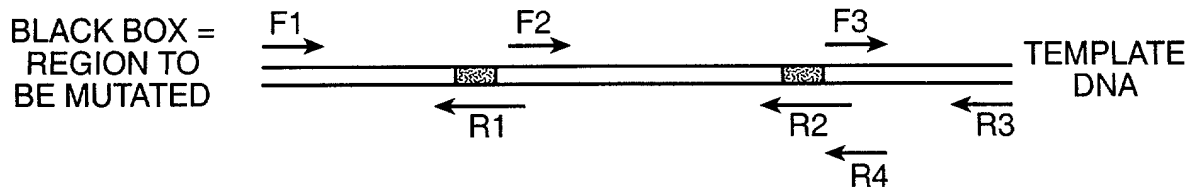


FIG._14



STEP 1: SET UP 3 PCR REACTIONS:

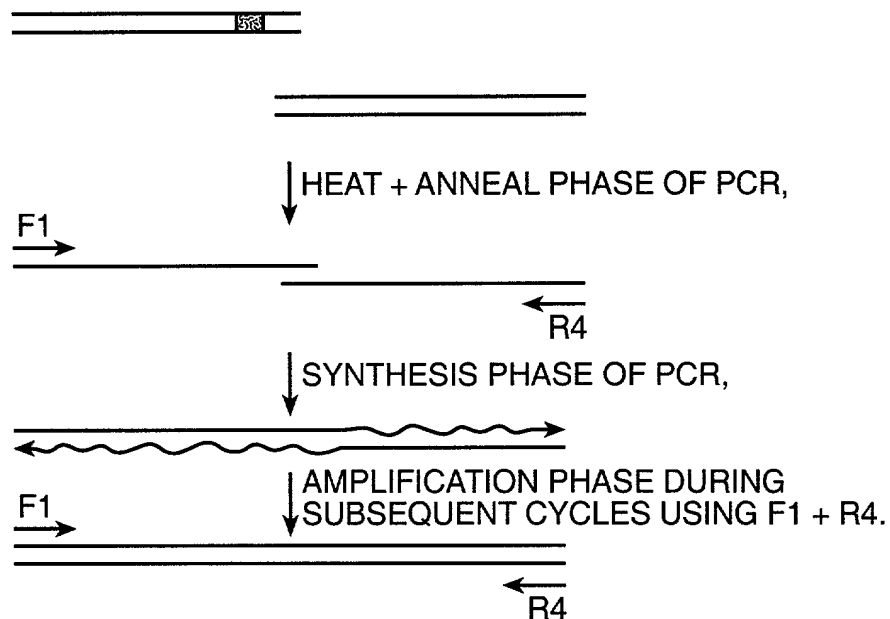
PRODUCTS:

TUBE 1:

TUBE 2:

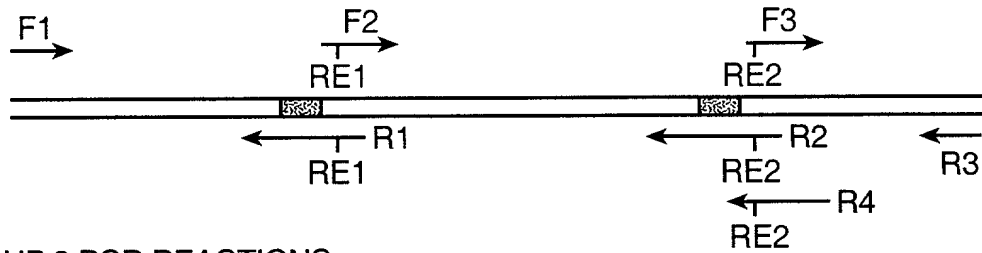
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.



STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG. 15



STEP 1: SET UP 3 PCR REACTIONS:

TUBE 1:
 A horizontal DNA template with a shaded rectangular restriction site labeled RE1. Below the template, primer R1 is indicated with an arrow pointing left.

TUBE 2:
 A horizontal DNA template with two shaded rectangular restriction sites labeled RE1 and RE2. Below the template, primers R1 and R2 are indicated with arrows pointing left. R1 is between the two sites, and R2 is between the second site and the right end.

TUBE 3:
 A horizontal DNA template with a shaded rectangular restriction site labeled RE2. Below the template, primer R2 is indicated with an arrow pointing left.

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG._16

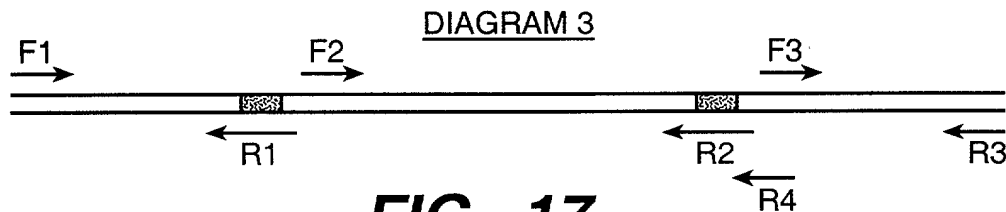


FIG._17